THE INFERTILITY OF RUDIMENTARY WINGED FEMALES OF DROSOPHILA AMPELOPHILA

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WHILE the infertility of the females of the mutant stock of *Drosophila* called rudimentary, was apparent from the beginning, the cause of the infertility was uncertain. Many rudimentary females bred to males of their own kind gave no offspring. The males with rudimentary wings, on the other hand, were perfectly fertile with wild females and with females of other stocks. The results might seem to show that sperm bearing the factor for rudimentary could not fertilize the eggs carrying the same factor. But that this was not the entire explanation was evident: for, heterozygous females fertilized by rudimentary males gave rudimentary females and males as well as long winged flies. In the heterozygous females, however, the egg, up to its maturity, has developed under the influence of the normal allelomorph of rudimentary, as well as of the rudimentary factor. I suggested,[1] therefore, that, due to this difference during the ripening period, the rudimentary bearing egg of the heterozygote could be fertilized by the rudimentary sperm, although the egg of the rudimentary female itself could not succeed in this combination, but I have never felt satisfied with this tentative explanation; for, there were other possibilities, not sufficiently studied, that might affect the result. For instance, it was not actually observed that the rudimentary males copulate successfully with females of their own kind, although it was known that they could mate with any other females. This question had first to be settled by direct observation.

Rudimentary winged females were isolated for three

[1] Morgan, *Zeit. f. indukt. Abs. und Vererb.*, VII, 1912.

days after hatching, and then each was mated to a rudimentary winged male that had similarly been isolated. In about twenty minutes, on an average, mating occurred in an entirely normal manner. The females that had mated were kept each in a separate bottle and given the best food. Examination showed that hardly one of the females had laid eggs; but in the rare cases where a few eggs were observed, some flies developed. In the first experiment seventeen females were seen to mate, and were then kept alone, or with their mates. One female produced one rudimentary winged son; another gave one rudimentary winged daughter and one such son. These three flies were the total output of seventeen females.

The next point was to determine whether these seventeen females were infertile only with their own kind of males. Each was again paired, this time to a male with bar eyes. The character bar eye is dominant. If sperm of these males should be successful, the female offspring from this cross would have bar eyes, and could be distinguished from any others that might come from the first mating. One female gave one bar daughter; another female also had one bar daughter. These results show that the rudimentary females were no more successful with bar males than with their own kind.

In a second experiment eleven rudimentary-winged females were tested with rudimentary males. One gave one rudimentary daughter and one rudimentary son, but also two long-winged daughters. Since I had not taken the same care here (using twenty-four-hour flies) as before to be certain that the females were virgin, these two long-winged daughters are supposedly due to fertilization before isolation, since long-winged males were hatching at the time in the parent stock. I tested this supposition by mating the two females to a rudimentary male, and obtained the following kinds of offspring:

Long ♀	Long ♂	Rudimentary ♀	Rudimentary ♂
56	64	15	23

Evidently then the rudimentary females had been fertilized by a long-winged brother before isolation, as well as by the rudimentary male later. No offspring were produced by a third mating, to bar males.

A third and similar experiment was made. Of twelve females, none gave any rudimentary offspring, two gave three bar daughters apiece, but no rudimentary sons.

In a fourth experiment thirteen rudimentary-winged females were isolated from stock. They were not necessarily virgins. One gave a rudimentary-winged son; another gave two rudimentary daughters and six such sons.

If only a single pair of flies is present in a culture and few or no larvæ are produced, the banana generally decays instead of fermenting. It might happen under these conditions that the few larvæ from rudimentary females might fail to develop, and the rudimentary sons might also suffer, even when some of their long-winged sisters (the father being a normal male) succeed in developing. To make conditions favorable in this respect I proceeded as follows: A red-eyed rudimentary female was kept at first with a red-eyed rudimentary male for three or four days. Then a few old white bar females and males were added and fresh food given. The presence of these flies and their progeny would serve to keep the food in good condition. Moreover if the rudimentary female had been fertilized by the rudimentary male she would produce rudimentary *daughters* and *sons*. If she were subsequently fertilized by the white bar males she would also give red (heretozygous) barred daughters, but these could not be distinguished from the daughters that the white-bar female would give were she fertilized by the red rudimentary males. Nevertheless all of the sons of a rudimentary female would be rudimentary round-eyed males, regardless as to which male was their father, and their presence would show to what extent the rudimentary females were fertile. The experiment was varied and simplified by removing the rudimentary males, when the white bar males and females were added.

One hundred and two rudimentary females were tested in these ways. In only three cases were rudimentary males produced. One female produced two; one female produced four, and another female produced one male. It is evident, therefore, that the scarcity of rudimentary sons can not, in general, be ascribed entirely to the conditions of the food.

As pointed out above, red bar daughters might appear in the foregoing tests and such females might have either of the two parentages specified. Most of the females of this kind would be expected to come from white bar females by rudimentary males, since the converse case would rarely be realized. Seven females, that appeared, were tested by breeding to white bar males and gave the results in the first of the two following tables. Four others were tested by breeding to rudimentary males with the result shown in Table I*a*. The results confirm the expectation

TABLE I

F_2, RED, LONG BAR (HETEROZYGOUS) ♀ BY WHITE BAR ♂

Red Long Bar ♀	White Long Bar ♀	Red Rud. Round ♂	White Long Bar ♂	Red Long Bar ♂	White Rud. Round ♂	Red Rud. Bar ♂	White Long Round ♀	Red Long Round ♂	White Rud. Bar ♂
55	44	13	24	27	7				
30	30	3	14	9	2				
65	61	3	27	10	5			2	
85	71	17	34	28	19				
48	53	15	27	21	17				
37	35	5	25	13	9				
12	10	1	5	5	0		1		1
342	304	57	156	113	59		1	2	1

TABLE I[a]

F_2, RED, LONG BAR (HETEROZYGOUS) ♀ BY RUDIMENATRY ♂

Red Long Bar ♀	Red Rud. Round ♀	Red Long Round ♀	Red Rud. Round ♂	White Long Bar ♂	Red Long Bar ♂	White Rud. Round ♂	Red Rud. Bar ♂	White Long Round ♂	Red Long Round ♂	White Rud. Bar ♂
22	15		14	13	14	6			1	
37	13	1	17	26	16	9				
65	10		9	26	25	24				
56	25		25	12	14	12				
180	63	1	65	77	69	51			1	

in regard to the character of these females. Three pairs of factors are involved which should give the following classes of males:

Non-cross-overs	*Single Cross-overs*	*Double Cross-overs*
Red, rud., round.	Red, long, bar.	Red, long, round.
White, long, bar.	White, rud., round.	White, rud., bar.
	Red, rud., bar.	
	White, long, round.	

It will be observed that the rudimentary males run far behind their schedules, due beyond doubt to their poor viability.

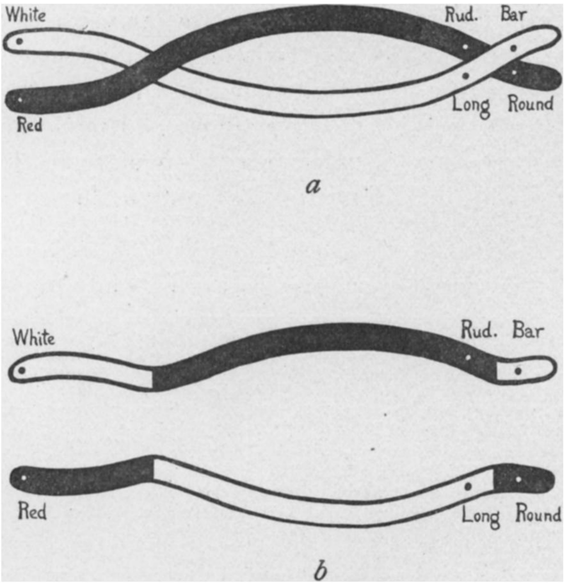

FIG. 1.

Double crossing-over took place four times in the experiment. If the two X chromosomes that carry respectively the factors for red rudimentary round and white long bar are represented as twisted once around each other, as in text-fig. 1, *a*, the result of fusion and recombination at the crossing points would give the two chromosomes shown in 1, *b*. One chromosome now carries the factors for red, long, round, and the other the factors for white, rudimentary, bar. As the tables show there are

four males that fall into these two classes. There is one female in the second table, that is red, long, round. She must have resulted from a cross-over gamete, a long, round egg being fertilized by a female producing sperm of the rudimentary male.

Still another experiment like the last one was made, but vermilion-eyed flies instead of bar-eyed flies were added. The virgin rudimentary females that were used were not allowed to mate first (as before) with rudimentary males, in order to meet a possible objection to the preceding experiment, namely that the spermathecæ, if first filled with sperm from the rudimentary males, might be incapable of filling again with sperm when another male of a different stock is added. The vermilion (long-winged males) would give, with their own females, flies with vermilion eyes, while the vermilion males that mated with the rudimentary females should give red-eyed, long-winged females and rudimentary red-eyed males. In the following table the number of rudimentary females tested in each culture is given in the top line; in the second, central, line the number of red, long offspring given by the flies in the square above; and in the lowest line a record of the number of vermilion offspring given by the flies in the culture under observation.

Number of rudimentary ♀ tested	1	6	1	1	1	7	6	1	6	5	4	6	15	6	4	4
Red long daughters		7				1	1								{5 ♀ 3 ♂	
Vermilion ♂ and ♀	18	463	76	119	105	121	264	637	233	28	6	54	179	0	167	46

Out of a total of seventy-four rudimentary females only nine red-eyed daughters were produced (if we exclude one culture in which five red long females and three red-eyed males appeared which must be due to error or to contamination). Of the nine offspring seven came from one culture, and possibly from one female in that culture that laid an exceptionally high number of eggs. The complete absence of rudimentary males may be explained

as a result of competition, for, as Morgan and Tice have shown, such males tend to disappear if too many other larvæ are present.

Lastly sixty-eight more rudimentary females from stock were tested with bar males. They gave twenty-four long bar (heterozygous) females, two rudimentary round males and one mosaic that will be described below. In one bottle there had been twenty-seven rudimentary females and an examination of their food showed over forty eggs present. Since the eggs are not easily found I estimate that probably a hundred eggs were present. Out of these eggs sixteen females and one male developed (included in the total given above). It appears then that many of the eggs laid by the rudimentary females do not develop.

The condition of the ovaries of the eight surviving rudimentary females showed that seven were full sized and contained mature eggs. The mosaic that appeared in one of the last crosses (Fig. 2) is interesting in several ways. Genetically it is a female, externally it is a male in appearance, in reality it is a male in part and a female in part although the egg must have been fertilized by a female-producing sperm. On the right side of the body the eye is heterozygous for bar, there is no sex comb on the fore leg, the spines on the thorax are long, and the wing is large. On the left side the eye is pure bar, there is a sex comb on the foreleg, the spines on the thorax are short, and the wing is small. The difference in size of the two wings, and of the spines, is a characteristic difference between the male and female, connected with a difference in body size. The abdomen is pigmented above as in the male, and below there is a normal penis.

Despite the apparently normal male copulatory organs, the mosaic, when placed with mature, unmated females, paid not the slightest attention to them, although it was quite active. Of course its organs of perception were female on one side of the anterior end, although male on the other side. What physiological complex this might give, is, of course, problematical. The mosaic died by

becoming stuck to the glass before its behavior towards males could be studied.

There are two ways in which this mosaic can be ac-

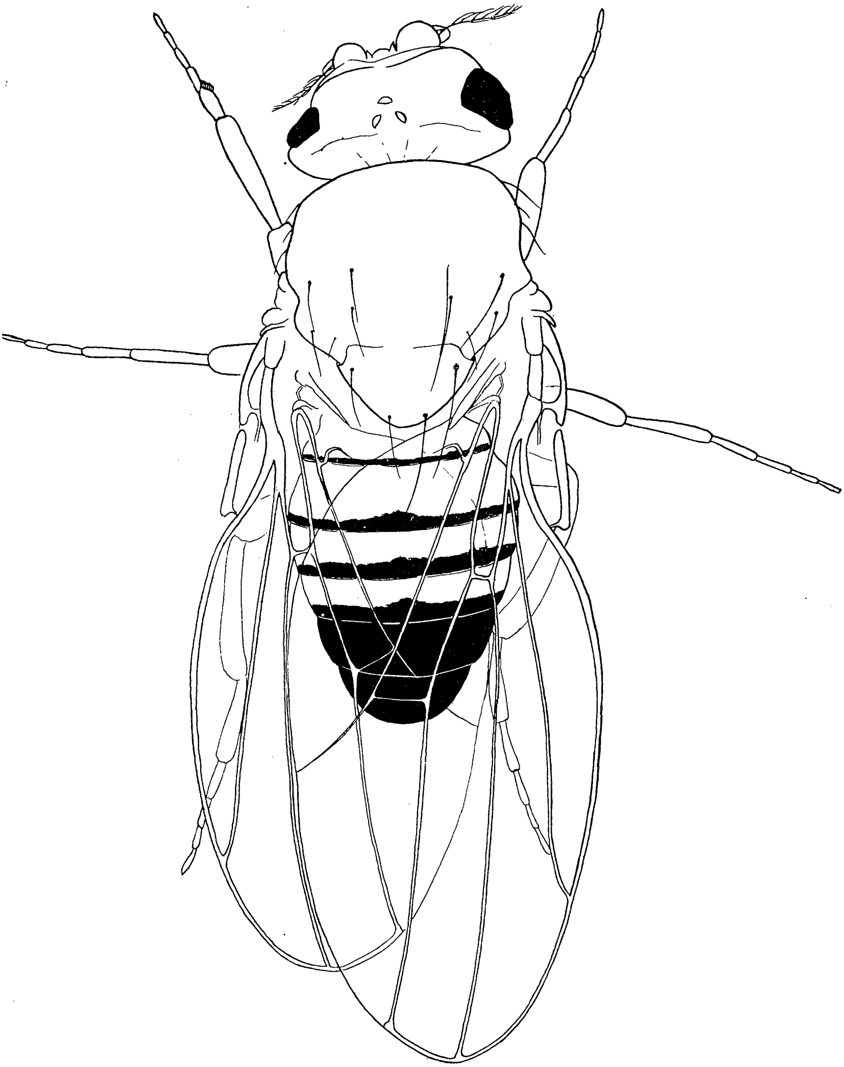

Fig. 2.

counted for. If an egg of the round-eyed, rudimentary female was fertilized by a female producing spermatozoon of the bar-eyed, long-winged male the result should

be a bar (heterozygous) eyed, long-winged female, since these are the dominants. If, then, after fertilization, dislocation of the X chromosomes occurred at some early division, so that while the X carrying the factor for bar and long divided normally (each daughter nucleus getting its proper half), the other X chromosome carrying the factors for round eye and long wings failing to divide (or else one half failed to go to one daughter nucleus) the characteristics of the mosaic can be accounted for. On the male side, the left, there would be one X chromosome in each cell, that carries the factors for bar eye and long wings. The size of the wing and of the spines, and the presence of the sex comb are a consequence of the "maleness," resulting from the presence of only one X. On the female side, the right, there would be two X chromosomes in all of the cells, hence the heterozygous nature of the bar eye. The length of the wing (the female being larger than the male) and the absence of the sex comb are a consequence of the "femaleness," due to the two X combination. The fact that the posterior end of the abdomen is purely male is owing to this region coming from the male contingent of nuclei, that must have overlapped to the right side in this region.

The other explanation of the mosaic is that two female producing nuclei entered, one alone giving rise to the male side, the other one uniting with the egg nucleus giving rise to the female side. Boveri's explanation of gynandromorphs will not apply to this case. There is no way to decide between the first two hypotheses, but, as I have shown elsewhere,[2] the hypothesis of chromosomal dislocation will cover all cases of gynandromorphs in *Drosophila*, while that of double fertilization will not apply to one case that gives, for itself at least, a crucial test of the alternative hypotheses. The hypothesis of chromosomal dislocation is, therefore, to be generally preferred, unless in some special case it can be shown that

[2] "Mosaics and Gynandromorphs in *Drosophila*," *Proc. Soc. Exp. Biol. and Med.*, XI, 1914.

double fertilization has actually brought about the particular results that that case shows.

Incidentally this sex mosaic (gynandromorph) and others of its kind confirm the conclusions drawn from grafting experiments in insects, namely, those in which the testes were grafted into the female and the ovary into the male without influence on the secondary sexual characters that developed later. These characters in the insects must be determined by the chromosomal composition of the cells, and not be affected by the sex "glands" as such. In contrast to this situation in the insects we find in birds that the sex "glands" of the female play an important rôle in the suppressing in the female of *some* of the secondary sexual characters—characters that appear only in the males or in castrated females. Gynandromorphs are exceedingly rare in birds, but there are a few well-authenticated cases. It is difficult to explain their occurrence under the conditions named above. It is just possible, however, that their occurrence may be accounted for in the following way. If a mosaic condition of the chromosomal complex should arise the secondary sexual characters would still all be like those of the female, owing to the presence of the ovarian secretion, but, if, in such a case, the ovary should become infected, or degenerate through senile changes, the true male parts might *sooner* develop the characteristics of the male than do the true female parts, *i. e.,* those parts of the body that have the female sex complex. This suggestion has no value unless it may lead some one to examine the condition of the ovary, when such a sex mosaic again appears.

An examination of the ovaries of many rudimentary females was made. In the majority of cases the ovaries become nearly as large as those in the normal female, and while they may contain full-sized eggs most of the eggs remain immature. Examination of the food shows that very few eggs are laid; in fact, most females lay no eggs. Of those laid some at least hatch. From these observations, and from the experiments, it seems clear that the

infertility of the rudimentary females is due, largely at any rate, to their retention of their eggs, even after copulation; and since in a few cases rudimentary females and males bred together have produced daughters as well as sons, the hypothesis of prematuration that I suggested in 1912 is not the correct explanation of the sterility of the females of the rudimentary winged stock mated to rudimentary males. Moreover, since many of the females tested, especially in the later experiments, were F_2's extracted through other fertile stocks, the sterility can not be supposed to be due to any additional peculiarity that has appeared in the rudimentary stock, but must be one of the attributes of the factor for rudimentary itself.